ADENOVIRUS-MEDIATED IN VIVO B7-1 GENE TRANSFER INDUCES ANTI-TUMOR IMMUNITY AGAINST PRE-ESTABLISHED PRIMARY TUMOR AND PULMONARY METASTASIS OF OSTEOSARCOMA IN A SYNGENEIC RAT MODEL

INTRODUCTION
Treatment of patients with osteosarcoma, especially those who have acquired resistance to current chemotherapy protocols, remains a major challenge. Active immunotherapy is a potential clue to overcome such therapeutic difficulties. Using a syngeneic rat model system, we previously demonstrated that exogenous expression of B7-1 costimulatory molecules in osteosarcoma cells induces curative as well as protective immunity against B7-1-negative parental osteosarcoma. In that model, an osteosarcoma cell vaccine was generated by ex vivo transfection of B7-1 cDNA into osteosarcoma cells. However, this strategy is not readily applicable to the clinical situations since development of osteosarcoma cell lines is rarely successful. More direct transfection strategies such as in vivo transfection using a viral carrier appear to be suitable. In the current study, we investigated the therapeutic efficacy of adenovirus-mediated in vivo B7-1 gene transfer on pre-established primary tumor as well as pulmonary metastasis of osteosarcoma.

MATERIALS AND METHODS
Rat B7-1 cDNA was constructed by RT-PCR. According to the COS-TCP method, the recombinant adenovirus vector encoding the rat B7-1 cDNA (Adex-B7-1) or LacZ (Adex-LacZ) was generated in 293 cells. Using these vectors and a rat osteosarcoma cell line, MSK-8G, (i) efficacy of in vitro as well as in vivo gene transfer into osteosarcoma cells and (ii) therapeutic efficacy of in vivo B7-1 gene transfer were evaluated. (i) MSK-8G cell culture was infected in vitro with Adex-B7-1. Forty-eight hours after infection, cell surface expression of the transfected gene products was determined by flow cytometry using anti-rat B7-1 mAb. In vivo gene transfer experiments, MSK-8G cells (1 × 10⁶) were inoculated into the right flank of syngeneic F344 rats. When subcutaneous tumors reached 8mm in the largest diameter, 5 × 10⁸ plaque-forming units (p.f.u.) of Adex-B7-1 or Adex-LacZ were injected intratumorally. On 3, 7, and 14 days after viral infection, subcutaneous tumors were resected and dispersed with collagenase. B7-1 expression on the dispersed cells was determined by flow cytometry. (ii) the therapeutic efficacy of in vivo Adex-B7-1 gene transfer was evaluated on pre-established primary tumor and pulmonary metastasis in two distinct models. First, MSK-8G cells (1 × 10⁶) were inoculated into F344 rats to develop a subcutaneous tumor, which was then treated with Adex-B7-1 or Adex-LacZ as described above. The tumor volume was weekly scored and calculated by length x width²/2. Four weeks after intratumoral injection of Adex-B7-1 or Adex-LacZ, the rats were again challenged with parental MSK-8G cells subcutaneously for assessing protective immunity. Specificity of cytotoxic T lymphocyte (CTL) activity was determined by the JAM test, using splenocytes as effector cells, which were obtained from the rats 9 weeks after adenoviral treatment, and MSK-8G and a F344 rat-derived gliosarcoma line, T9, as target cells. Second, MSK-8G cells (1 × 10⁶) were injected into the tail vein and the subcutis of rats concomitantly to establish pulmonary metastasis and a primary tumor, and 5 × 10⁸ p.f.u. of Adex-B7-1 or Adex-LacZ was injected intratumorally when subcutaneous primary tumors reached 8mm in the diameter. The number of metastatic nodules in the lung was counted 3 weeks after adenoviral treatment. Five rats were used in each experiment. Student t-test was used for statistical analysis and a probability of less than 0.05 was accepted as significant.

RESULTS
Flow cytometric analysis revealed that more than 90% of MSK-8G cells infected in vitro with Adex-B7-1 expressed B7-1 molecules in the cell surface. In vivo transfer of Adex-B7-1 resulted in persistent expression of B7-1 in MSK-8G cells for at least 2 weeks. Intratumoral injection of Adex-B7-1, but not Adex-LacZ, apparently regressed the volume of the subcutaneous MSK-8G tumors, with complete tumor rejection in 3 out of 5 rats (Figure). An additional challenge of MSK-8G cells failed to form tumors in Adex-B7-1-treated rats, whereas no such protection but progressive tumor growth was observed in Adex-LacZ-treated rats. Splenocytes prepared from Adex-B7-1-treated rats preferentially lysed MSK-8G cells. The number of pulmonary metastatic nodules in Adex-B7-1-treated rats was 20 +/- 12.2, which was significantly smaller than that in Adex-LacZ-treated rats (46 +/- 21.5) or rats with no treatment (60.5 +/- 10.9).

DISCUSSION
In the present study, we found that adenovirus-mediated B7-1 gene transfer induces (i) expression of B7-1 molecules in osteosarcoma cells by both in vitro and in vivo infection procedures, (ii) curative immunity against pre-established primary osteosarcoma, which lasts for at least 4 weeks to protect against the additional challenge of parental B7-1-negative osteosarcoma cells, and (iii) systemic immunity against pre-established pulmonary metastasis. The specificity of the induced immunity to osteosarcoma cells was confirmed by CTL assay using a syngeneic gliosarcoma cell line. These findings support the costimulatory role of B7-1 in induction of anti-tumor immunity against osteosarcoma and also the usefulness of adenovirus as a carrier of gene transfer.

REFERENCES

**Division of Molecular Immunology, Institute of Genetic Medicine, Hokkaido University, Sapporo, Japan.**